ELSEVIER

Biochimica et Biophysica Acta, 1365 (1998) 175–184

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Improved isolation and crystallization of Photosystem I for structural analysis

Petra Fromme*, Horst Tobias Witt

Max Volmer Institut für Biophysikalische Chemie und Biochemie, Technische Universität Berlin, Strasse des 17. Juni 135, D-10623 Berlin, Germany

Received 18 February 1998; accepted 23 February 1998

Abstract

Stable trimeric Photosystem I (PS I) was isolated from the cyanobacterium *Synechococcus elongatus* by use of stereochemically pure β-dodecylmaltoside. Crystals of extremely pure PS I are grown by dialysis against low salt concentration. The improved PS I crystals were the basis for a PS I model derived from X-ray structure analysis at 4 Å resolution (Krauß et al., Nat. Struct. Biol. 3 (1996) 965–973; Schubert et al., J. Mol. Biol. 272 (1997) 741–769) and for detailed information on the electron transfer chain elaborated by techniques of magnetic resonance (H. Käß, Thesis, TU–Berlin, 1995; Kamlowski et al., Biochim. Biophys. Acta 1319 (1997) 188–198 and 199–213; Bittl et al., Biochemistry 36 (1997) 12001–12004). Crystallization procedures using micro- and macroseeding are described as basis for the forthcoming structure analysis. © 1998 Elsevier Science B.V.

Keywords: Photosystem I; Photosynthesis; Crystallization; Structure analysis; Membrane protein

1. Introduction

The primary step in oxygenic photosynthesis, the light induced transmembrane charge separation, takes place in plants and cyanobacteria in two large membrane intrinsic protein complexes, the Photosystems I and II (PS I and PS II) (for an overview on photosynthesis see Ref. [1]). PS I catalyses the light driven electron transfer from plastocyanin/cytochrome c_6 at the lumenal side to ferredoxin at the stromal side of the thylakoid membrane by a transmembrane chain of six spectroscopically identified electron carriers P700 (a chlorophyll *a* dimer), A0 (a chlorophyll *a* monomer), A1 (a phylloquinone) and three Fe_4S_4 clusters, F_X, F_A and F_B. PS I of cyanobacteria consists of 11 protein subunits (A–F and I–M), about 100 chlorophyll *a* molecules 20–25 carotenoids, three [4Fe4S] clusters and two phylloquinones (for reviews on PS I see Refs. [2,3]). Electron microscopy of PS I isolated from the cyanobacterium *Synechococcus elongatus* showed a

*P.F. and H.T.W. are both corresponding author. E-mail: fromme@phosis1.chem.tu-berlin.de; witt@phosis1.chem.tu-berlin.de

Abbreviations: A0, spectroscopically identified first electron acceptor; A1, spectroscopically identified second electron acceptor; β-DM, β-*n*-dodecylmaltoside; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; F_X, F_A and F_B, iron sulfur clusters; HPLC, high pressure liquid chromatography; MES, morpholine-ethanesulfonic acid; P700, primary electron donor; PS I, Photosystem I; Q_K, phylloquinone electron acceptor; SB 12, sulfobetaine 12; SDS, sodium dodecyl sulfate.

PII: S0005-2728(98)00059-0

trimeric structure [4,5], The trimer was separated into three monomers each of which contains one P700; therefore the monomer represents one functional unit of PS I [6], The genes encoding the 11 PS I subunits of this cyanobacterium were isolated and sequenced [7].

Crystals, suitable for X-ray structure analysis, were obtained from the cyanobacterial trimeric PS I [8] and grown by dialysis against low salt concentration [9]. This was the basis for the first model of PS I, based on X-ray structure analysis at 6 Å resolution [9,10].

In the following, progress in isolation and crystallization of PS I is described as the basis for the PS I X-ray structure analysis at 4 Å resolution [11,12] as well as for the investigations of electron carriers by methods of magnetic resonance.

2. Isolation of PS I

2.1. Cell growth and thylakoid preparation

Cells from the thermophilic cyanobacterium *S. elongatus* [13] were grown in two 30 l reactors at 56°C (for medium see Ref. [14]). The cultures are supplemented with air enriched with 4% CO_2 (flow-rate 50 l/h) and are grown at continuous light. Eighty g cells can be obtained in 1 week.

The cells were washed in buffer A (20 mM morpholine-ethanesulfonic acid (MES)–Na pH 6.4, 10 mM $MgCl_2$ and 10 mM $CaCl_2$) and resuspended in buffer B (the same as buffer A with addition of 500 mM mannitol). The cell walls were degraded using 0.1% lysozyme (1 h incubation time in the dark at 43°C). Cells were broken at 4°C using a Yeda Press. Thylakoids were collected by centrifugation and washed twice with buffer A. In order to remove membrane attached proteases the thylakoids were washed once with 0.2 M KCl in buffer A.

2.2. Extraction and purification of PS I

In order to solubilize PS I, the thylakoids (chlorophyll concentration 1 mM) were incubated with β-dodecylmaltoside (β-DM) (α-isomer content <0.1%[1]) (final concentration 0.6%) at 20°C for 20 min in the dark. The thylakoids were separated from the extract by ultracentrifugation. All extracts were characterized by sodium dodecyl sulfate (SDS) gel electrophoresis (Pharmacia, Phast System, HD gel system, silver stain). HPLC anion-exchange chromatography (Mono Q, Pharmacia) was used to determine the ratio of PS I monomer/PS I trimer and PS II in the extracts as described elsewhere [6]. Only extracts with more than 60% trimeric PS I were used for the further purification steps.

In order to separate phycobillisomes from the photosystems the extracts were directly applied to the anion-exchange column Q-Sepharose HP, running with buffer C50 (CX: 20 mM MES pH 6.4, 0.02% β-DM, X mM $MgSO_4$) at 4°C. Sucrose density-gradient centrifugation, used in the former protocol, was omitted to prevent partial dissociation of trimers into monomers during column chromatography [15]. Increasing the salt concentration to 100 mM leads to elution of the main part of PS I monomer and PS II. Trimeric PS I was finally eluted by increasing the salt concentration to 150 mM $MgSO_4$. The solution was diluted with buffer C0 to 70 mM $MgSO_4$ and applied to a HPLC Q-Sepharose column running with a linear gradient from 70 to 170 mM $MgSO_4$ in buffer C. Trimeric PS I elutes in a sharp peak at a 150 mM $MgSO_4$. One hundred and twenty mg of the PS I protein complex were isolated from 80 g cells. Trimeric nature and purity of protein were proofed by

[1]The purity of the β-DM is critical for the final resolution limit of the PS I crystals. It is difficult to obtain this ultra pure detergent commercially, because even different charges from the same company vary extensively in purity (for example, β-DM charges from Boehringer vary between 0.05–7.0% α-isomer). Therefore test probes of different charges were ordered from different companys [Boehringer, BioMol and Anatrace (USA)] and the content (α/β-isomer) analyzed by HPLC cromatography: for the separation a C_{18} column [Eurospher 100-C_{18} 5 μm (l=250 mm ∅=4 mm; Knauer)] was used with a Knauer HPLC System. Seventy-two percent HPLC-grade methanol in water (also HPLC grade) (w/w) was used as fluid phase with a flow-rate of 1 ml/min. β-DM was dissolved at a concentration of 0.1 M in 72% methanol and 20 μl were injected/run. α-DM was used at the same concentration as a standard. The peaks were detected by an refractive index detector. Retention times of 24 min for the α-isomer and 26 min for the β-isomer of DM are observed. Using this procedure a content of ≥0.05% α-isomer can be reproducibly detected.

HPLC gel filtration (TSK 4000 SW) as described in Ref. [6]. The subunit composition was analyzed by SDS gel electrophoresis (see Fig. 1). The subunits, known to constitute cyanobacterial PS I (see above), were identified in the protein solution as well as in the crystals.

Using the new preparation protocol, the quantity as well as the quality of the PS I preparation was improved:

In the former isolation protocol [6] PS I was extracted from the membrane with the detergent

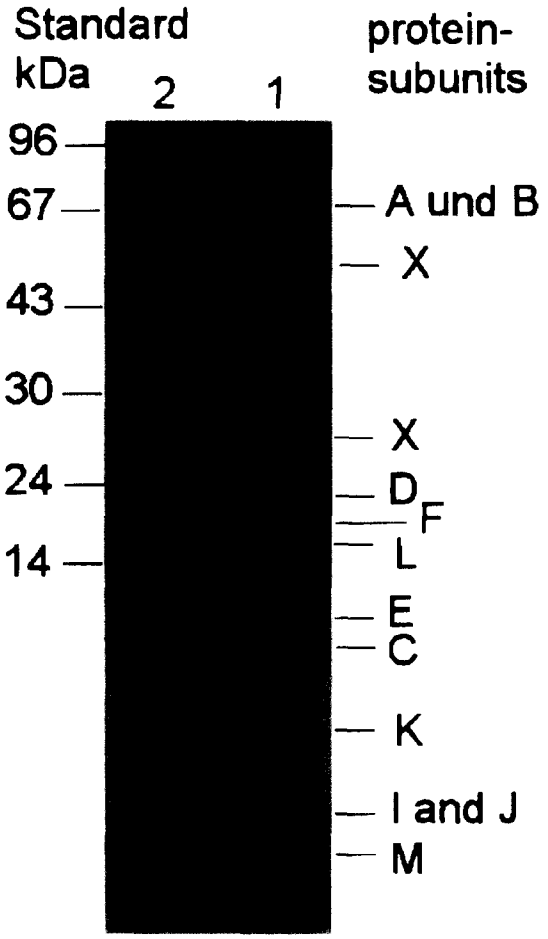

Fig. 1. SDS gel electrophoresis of the isolated crystalline Photosystem I complex. Lane 1, Photosystem I after the first crystallization step; some minor impurities (X) are still visible; Lane 2, homogeneous Photosystem I after the second crystallization step. Assignment of the single subunits was performed for subunits A,B,C,D,E,K and M by N-terminal sequencing [7], subunits F,L and I were identified by antibodies, which were a gift from P. Chitnis. SDS gel electrophoresis was performed using the Phast System of Pharmacia (Sweden) with the 'High Density' gel system. The SDS gel was stained with silver according to the procedure suggested by Pharmacia. Molecular weights of protein standards are indicated on the left.

sulfobetaine 12 (SB 12), and β-DM was used for all further purification steps. Now, stereochemical pure β-DM is used exclusively for the solubilization of the trimeric PS I complex as well as for all purification steps. There is some indication that the use of the zwitterionic detergent SB 12 could have induced a partial loss of subunit F, which therefore seemed to be 'disordered' in the 6 Å electron density map [10]. In the structural model of PS I at 4 Å resolution we found that subunit F is involved in the contacts between the PS I molecules within the crystals; the use of β-DM instead of SB 12 leads to improved ordered crystals.

The purity of the detergent is also important for the quality of crystals. If the detergent β-DM contains more than 2% of the α-isomer, no crystals can be observed; instead phase separation in two liquid phases occurs. PS I is concentrated in the detergent rich phase; no crystals can be grown under these conditions. Using detergent of an α-isomer content between 2% to 0.5%, crystals can be obtained but the resolution of the crystals is anisotropic: the resolution in the membrane plane (crystallographic *a/b* plane) is limited to 5–6 Å, whereas perpendicular to the membrane plane (crystallographic *c* plane) diffraction to resolution of 3.5 Å can be observed. The resolution in the *a/b* plane increases with the stereochemic purity of the detergent, so that the anisotropy of the X-ray diffraction is reduced and the resolution of the diffraction in the *a/b* plane is improved to even 3.5 Å by use of stereochemical pure β-DM with an α-maltoside content below 0.1%.

The homogeneity of the PS I preparation with respect to the quaternary structure is essential for the crystallization of the trimeric[2] PS I. Test experiments showed that monomeric PS I hinders the growth of large, well ordered PS I crystals at a ratio of one monomer to 10 000 trimers. This may be due to the fact that the subunits E and F, which are forming the interaction sites within the crystal, are present in trimeric PS I as well as in monomeric PS I. Since the trimeric axis is a crystallographic axis, crystal growth is inhibited at the sites where monomeric PS I is bound.

[2]PS I may exist in the photosynthetic membrane both in trimeric and in monomeric form [16]. Both forms can be extracted with detergent from thylakoid membranes.

3. Crystallization of PS I

The crystals of PS I suitable for structural analysis are grown in a salt concentration range where the protein solubility is reduced by decreasing the salt concentration ('reverse of salting in') [9]: in a first step at ca. 6 mM $MgSO_4$, slightly depending on the protein concentration, large amounts of small crystals (<0.1 mm) grow by spontaneous nucleation within minutes. These were used for a last purification step and resolubilized by increase of the salt concentration to ≈50 mM. Subsequently, in a second final step, the salt concentration was again diluted to the critical crystallization point at ca. 6 mM salt (4°C; pH 6.5) but slowly in a controlled way in a dialysis machine [9,17]. Within 1–2 days suitable hexagonal crystals of ≈1–2 mm length were obtained, which were used for X-ray structure analysis at 6 Å resolution [9,10].

Formation of small crystals is used as a last purification step also for the improved crystallization. The PS I solution is concentrated by ultrafiltration to a concentration of 10 mM chlorophyll. Subsequently, buffer D0 (DX: 5 mM MES pH 6.4 and 0.02% β-DM, X mM $MgSO_4$) is added (final concentration 9 mM $MgSO_4$). The critical $MgSO_4$ concentration is 9 mM instead of 6 mM, because PS I, isolated according to the new protocol, shows a significant lower solubility. This solution is concentrated to 10 mM chlorophyll by ultrafiltration, whereby large amounts of small crystals grow by spontaneous nucleation. The small crystals were washed with buffer D6 and buffer D0.

A second resolvation and a batch crystallization step ($MgSO_4$ concentration 6 mM; chlorophyll concentration 1 mM) is introduced for further purification. The total yield for the two crystallization steps is 40–60% of the purified protein. The crystals can be stored at 4°C in buffer D0 without addition of azide or protease inhibitors for several months.

Instead of using the dialysis machine, the final crystallization steps were performed in quartz tubes (inner diameter 3 mm), sealed with a dialysis membrane (BRL) with a cut off of 12 kDa. (The quartz tubes can be autoclaved and the volume of protein solution can be reduced to 4–20 μl).

PS I was crystallized by dialysis against low salt concentration as described above. Nucleation was induced by heterogenous seeding using hair and/or wool. Due to the higher purity and homogeneity of the protein the resolution of structural analysis increased from 6 Å [9,10] to 4 Å [11,12].

3.1. Micro- and macroseeding

In order to further increase the quality of the crystals for forthcoming structure analysis, homogeneous seeding techniques (micro- and macroseeding) were used. Crystallization of PS I was performed in three steps, indicated in Fig. 2, showing the schematic phase diagram of the solubility of PS I as a function of salt concentration.

1. In the first step, small PS I crystals (diameter ≈0.05 mm, length ≈0.1 mm) were grown by spontaneous nucleation (see above). The protein solution (PS I [10 μM]) was brought into the nucleation zone either by decreasing the salt or increasing the protein concentration. In this step, large amounts of small PS I crystals grow over night. They are shown in Fig. 3A.
2. In the second step, the small PS I crystals were used for microseeding. The protein solution (PS I [100 μM]) was brought into the metastable zone by decreasing the salt concentration by microdialysis. After one day of equilibration, 0.5 μl of a solution containing the microcrystals was added and over a period of two days the salt concentration was further reduced by microdialysis to the final concentration[3]. In this step 10–20 medium size crystals (diameter ≈0.1 length ≈0.1–0.3 mm) grow within 2 days. They are shown in Fig. 3B.
3. The third crystallization step uses the medium size crystals for macroseeding. The salt concentration of the protein solution (PS I [200 μM]) was slightly reduced to a concentration, at which the solution is still unsaturated but close to border of the saturation curve. When the solution has been equilibrated for 1 day, one single crystal of medium size is added. Dialysis against lower salt concentration is started immediately after addition. First, the crystal begins to dissolve, but due to the parallel decrease of the

[3]For the use of these seeding techniques the phase diagram has to be known quantitatively. The solubility was determined as a function of the salt concentration, the pH, and the temperature (Jordan et al., in preparation).

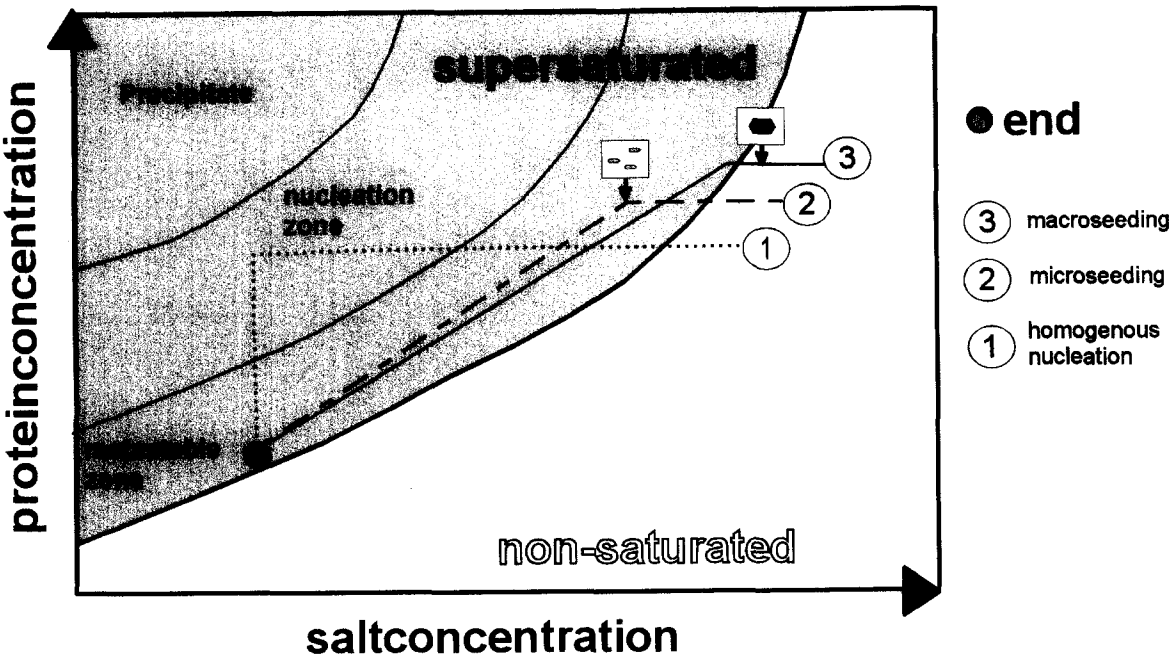

Fig. 2. Schematic phase diagram for the crystallization of trimeric Photosystem I from *Synechococcus elongatus* by spontaneous nucleation, microseeding and macroseeding through dialysis against low salt concentration. Crystallization is performed in three steps: (1) spontaneous nucleation, at high supersaturation large amounts of small crystals are formed; (2) microseeding, the protein solution is brought into the metastable zone, nucleation is induced by addition of a solution containing some small crystals (addition is indicated by an arrow), medium size crystals grown from this solution; (3) macroseeding, the protein solution is brought close to the saturation limit, one single crystal of medium size is added (indicated by an arrow) and the salt concentration is further decreased by dialysis leading to the growth of one large single crystal (the dotted, dashed and solids curves indicating the course of crystallization are chosen schematically).

salt concentration the solution becomes supersaturated and the growth of the crystal is observed. Using this procedure one large single crystal grows within 2–3 days, mostly hexagonal needles (diameter 0.5 mm, length 2–3 mm), less frequently also hexagonal plates can be observed (Fig. 3C).

Recently, crystals diffracting X-rays to 3 Å resolution have been observed (see Fig. 4), when this seeding procedure was used. A native data set was measured to 3 Å resolution under cryogenic temperatures at the Synchrotron at ESRF in Grenoble. Evaluation of these data is in progress (Jordan, Krauß, Saenger, personal communication).

4. Structural analysis based on the crystals of PS I

The 4 Å PS I crystals constitute the basis for several independent ways for structural and functional investigations (as shown in Fig. 5).

The X-ray structure analysis at 4 Å resolution [11,12] shows the main structure elements of PS I. Forty-three alpha helices were identified and assigned to the protein subunits in PS I, taking into account results from crosslinking work [2,18] as well as results from electron microscopy [19,20] (see Fig. 5A). The inner core of PS I (consisting of the five C-terminal helices of each of the large subunits A and B) shows a strong structural similarity to the structure of the purple bacterial reaction center [21–25]. A detailed comparison of the structure of PS I with the other photoreaction centers, including PS II [26] shows that the type 1 reaction centers (as PS I and reaction centers of green sulfur bacteria and heliobacteria) and the type 2 reaction centers (as PS II and purple bacterial reaction centers) have evolved from a common ancestor, as suggested earlier [27–29].

With respect to the chlorophylls, a total of 89 chlorophylls were identified and localized. Eighty-three chlorophylls set up the unique antenna system

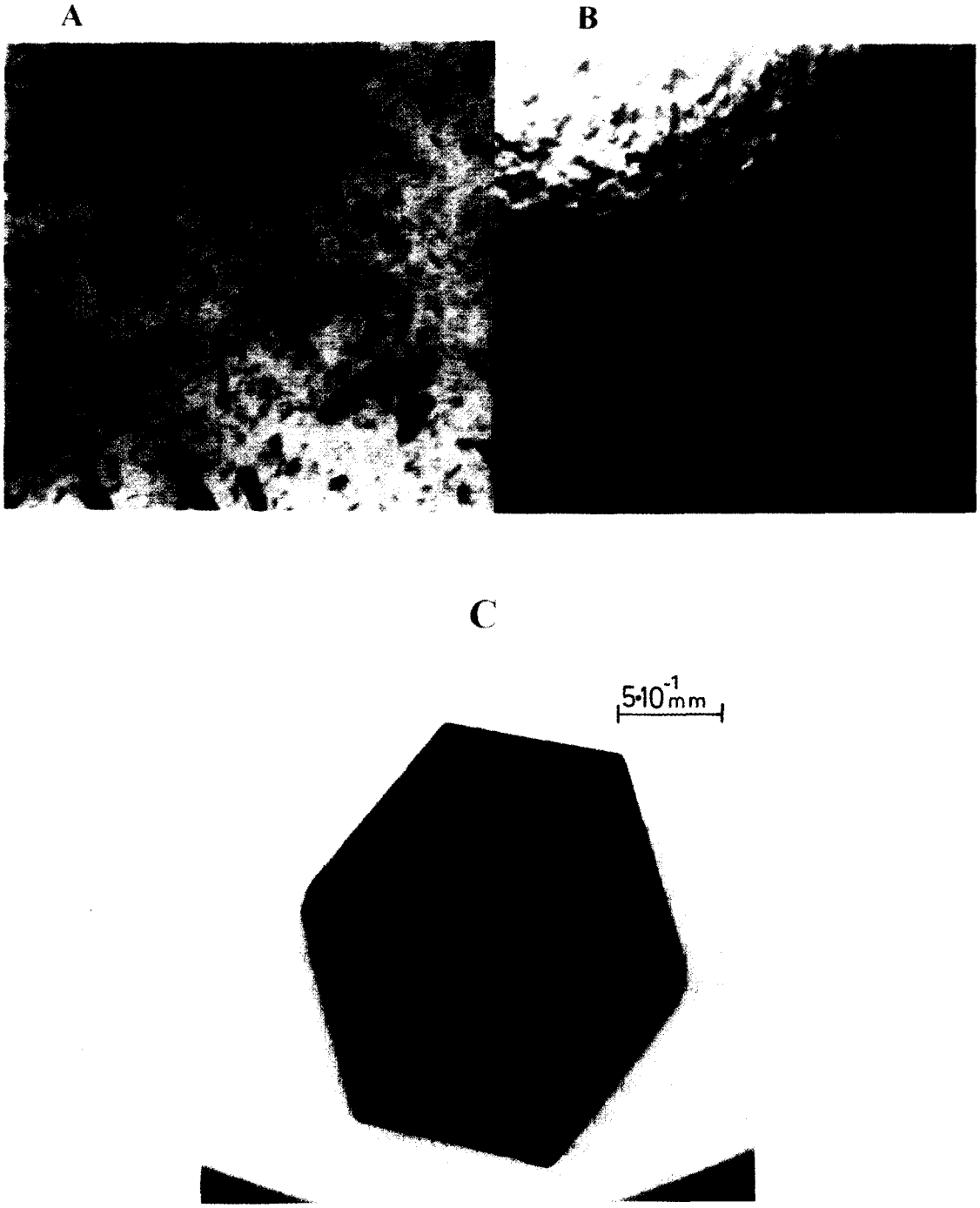

Fig. 3. Photographs of Photosystem I crystals: (A) small crystals diameter <0.05 mm, length <0.1 mm; (B) medium size crystals diameter ≈0.1 mm, length 0.1–0.3 mm; (C) large single crystal diameter 1.5 mm, height of the plate 0.3 mm.

of PS I and six chlorophylls were assigned to the electron transfer chain.

Detailed information on the electron carriers came from magnetic resonance investigations of PS I single crystals:

P700, the primary electron donor, has been iden-

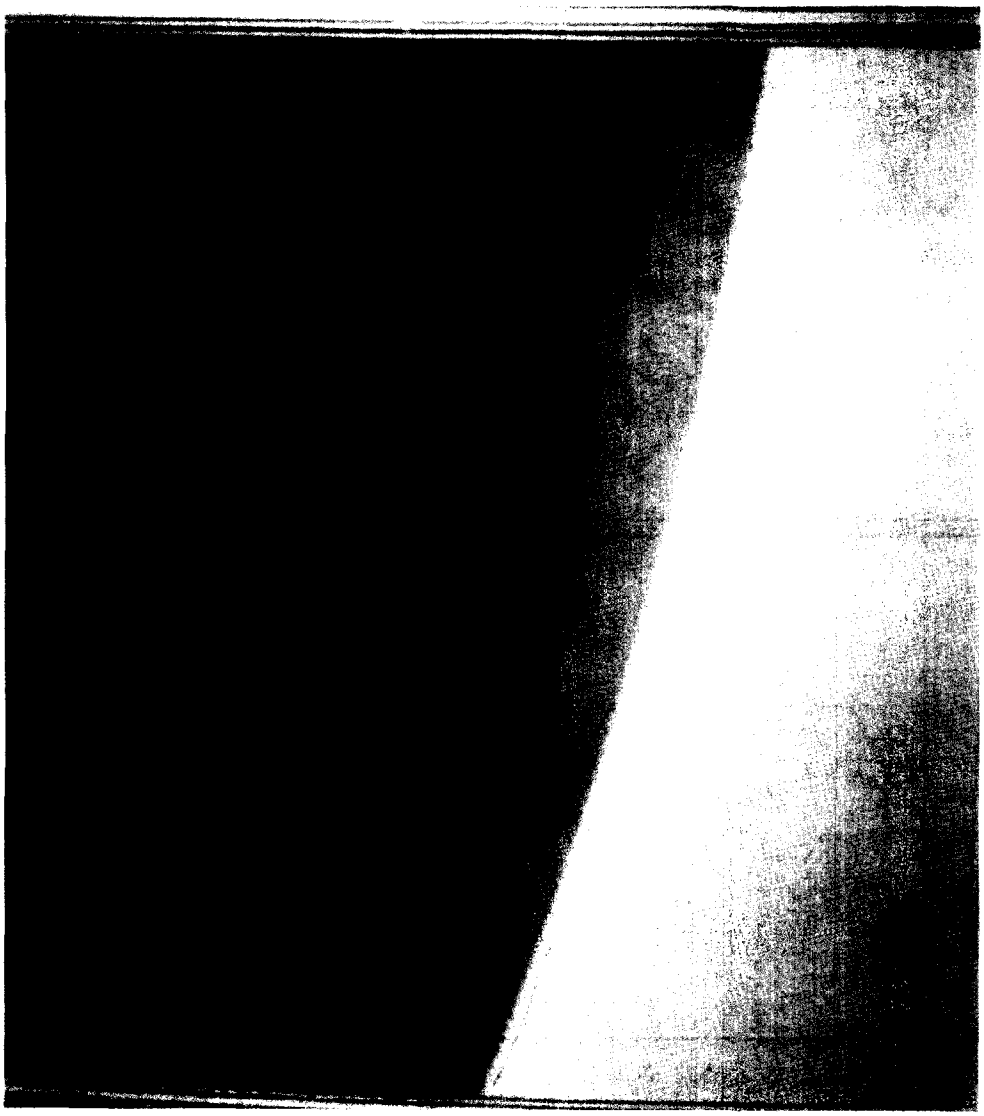

Fig. 4. Section of a diffraction pattern of Photosystem I crystals obtained by macroseeding and measured at the high brillinace beamline ID 2 at ESRF (Grenoble) $\Delta\varphi$/image, d=430 mm, λ=0.99 Å. The edge of the plate corresponds to a resolution of 3 Å.

tified as a dimer in the X-ray structural model. The hyperfine structure of the oxidized primary electron donor $P700^{+\cdot}$ was investigated by electron nuclear double resonance (ENDOR) experiments ([30]; Käß et al., in preparation). It was found that the spin density distribution is asymmetric; i.e., more than 85% of the spin density is located on one of the two chlorophylls of the P700 dimer. Also the circular orientation of the spin carrying chlorophyll within its plane was determined by ENDOR by the interaction of the spin of the unpaired electron of $P700^{+\cdot}$ with the spins of the protons of the methyl groups 7 and 12 as a function of the orientation of the crystal. The model derived from these investigations is shown in Fig. 5B.

Detailed electron paramagnetic resonance (EPR) investigation of the single crystals led to information concerning the terminal FeS clusters in PS I, F_A and F_B, which are bound to the extrinsic subunit C [31,32]. The position of the FeS clusters in the PS I complex was determined by their high electron density [10–12], but the orientation of the clusters

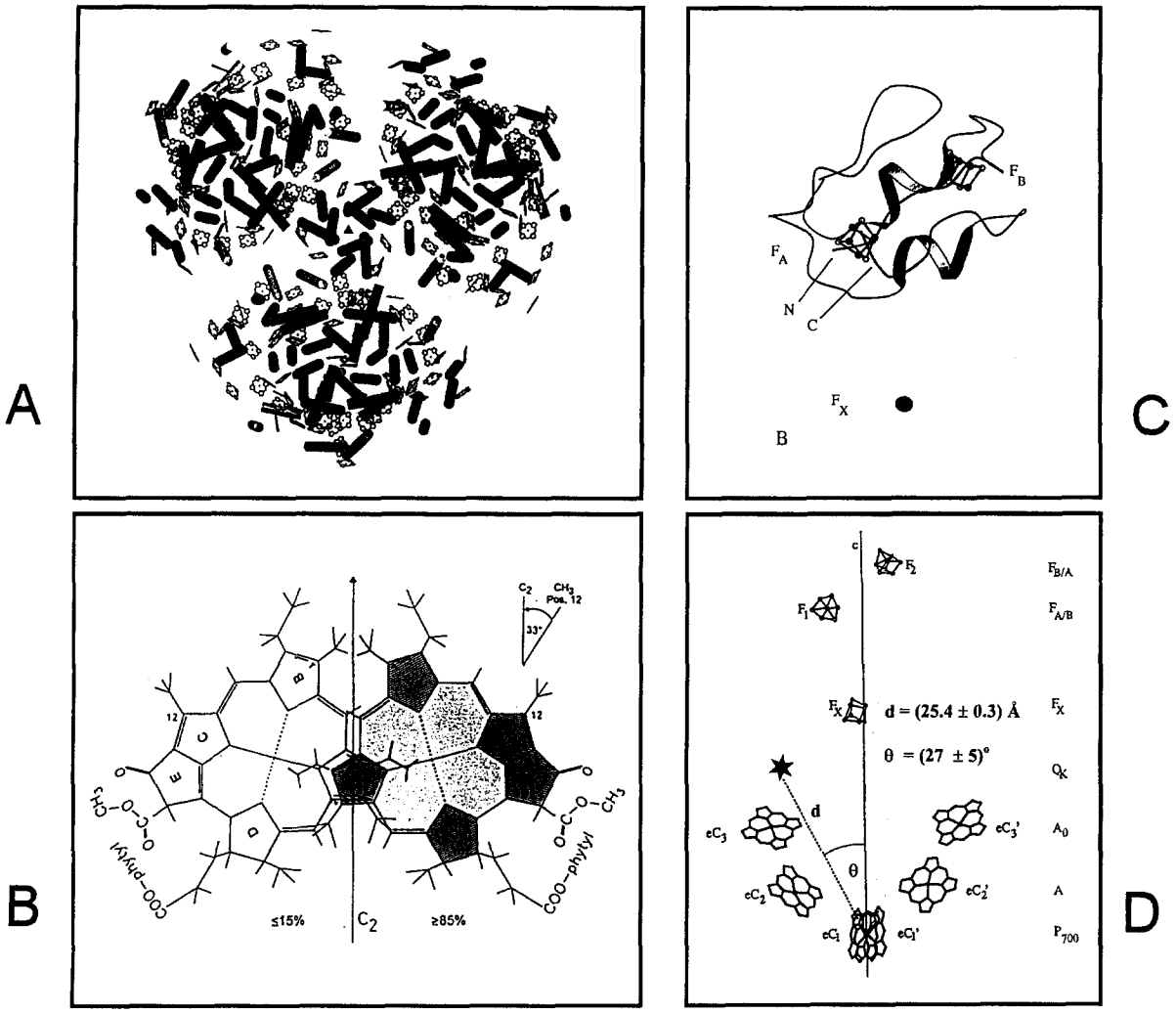

Fig. 5. Photosystem I crystals as the basis for different structural investigations. (A) Structural model of the trimeric Photosystem I derived from X-ray structure analysis at 4 Å resolution (from Ref. [12]). The alpha helices are shown as columns, the chorophylls are represented by the dihydroporphyrin-head groups and the FeS centers are shown in the form of open cubes. (B) Model of P700 derived from ENDOR investigation on single crystals of Photosystem I [30], reviewed in Ref. [17] (for details see text). Right: circular orientation of the chlorophyll *a*, which carries more than 85% of the spin of the unpaired electron. Left: possible orientation of the second chlorophyll *a* as derived from similarities to the primary donor of the purple bacterial reaction center; C2 is the local twofold axis; (lumen on top). (C) Structural model of subunit C and the FeS clusters F_A and F_B, which are coordinated by these subunits as derived from the combination of EPR investigations of single crystals [33,35] and the results of the X-ray structure analysis (from Ref. [35]). (D) Position of the phylloquinone electron acceptor (Q_K) determined by puls EPR on single crystals of Photosystem I [38] in relation to the position of the other electron carriers, determined by X-ray structure analysis [11] (figure slightly modified from Ref. [38]); in (C) and (D) stroma on top.

was arbitrary in the X-ray structural model. The positions and orientations of both clusters were determined in relation to the crystal axis by EPR investigations [33]. It was shown that subunit C shows strong structural homology to the soluble bacterial ferredoxins [34]. Together with the results of the X-ray structure analysis this leads to a detailed model of the structure of the subunit C [35]. One of the two rotational symmetrical models is shown in Fig. 5C.

Most of the electron carriers were assigned in the electron density map at 4 Å resolution (see above), but the two phylloquinone electron acceptors (Q_K) could not be identified in the electron density map due to the limited resolution of 4 Å. Therefore puls EPR investigations were performed, which led to the

determination of the distance of $P700^{+\cdot}$–$Q_K^{-\cdot}$ at (25.4±0.3) Å [36,37]. From puls EPR measurements on single crystals of PS I the angle between the $P700^{+\cdot}$–$Q_K^{-\cdot}$ connecting vector and the crystallographic axis was determined (27±5)° [38]. Together with the distance of $P700^{+\cdot}$–$Q_K^{-\cdot}$ the position for $Q_K^{-\cdot}$ was determined. This coincidences with a pocket of electron density tentatively assigned to Q_K [12].

Taking all results into account a detailed model of PS I has been obtained. Further spectroscopic investigation as well as an improved X-ray structure analysis based on the 3 Å cryo-data sets may lead to further progress in the understanding of the structure and function of PS I.

Acknowledgements

We thank H. Schmidt, D. Difiore and C. Lüneberg for the excellent technical assistance. Our thanks are to P. Chitnis for providing specific antibodies. We want to thank all collaborators on the structural investigations for the fruitful cooperation and the permission to use some of our published figures in this article. We thank H. Käß and W. Lubitz for the disposal of the model of P700 prior to publication. This work was supported by the Deutsche Forschungsgemeinschaft Sonderforschungsbereich 312 gerichtete Membranprozesse and by the Fonds der chemischen Industrie.

References

[1] H.T. Witt, Ber. Bunsenges. Phys. Chem. 100 (1996) 1923–1942.
[2] P.R. Chitnis, Q. Xu, V.P. Chitnis, R. Nechustai, Photosynth. Res. 44 (1995) 23–40.
[3] J.H. Golbeck, in: D. Bryant (Ed.), Advances in Photosynthesis. Vol. 1. Molecular Biology of Cyanobacteria, 1994, pp. 319–360.
[4] E.J. Boekema, J.P. Dekker, M.G. van Heel, M. Rögner, W. Saenger, I. Witt, H.T. Witt, FEBS Lett. 217 (1987) 283–286.
[5] E.J. Boekema, J.P. Dekker, M. Rögner, I. Witt, H.T. Witt, M. van Heel, Biochim. Biophys. Acta 974 (1989) 81–87.
[6] M. Roegner, U. Mühlenhoff, E.J. Boekema, H.T. Witt, Biochim. Biophys. Acta 1015 (1990) 415–424.
[7] U. Mühlenhoff, W. Haehnel, H.T. Witt, R.G. Herrmann, Gene 127(1) (1993) 71–78.
[8] I. Witt, H.T. Witt, D. DiFiore, M. Rögner, W. Hinrichs, J. Granzin, C. Betzel, Z. Dauter, Ber. Bunsenges. Phys. Chem. 92 (1988) 1503–1506.
[9] H.T. Witt, N. Krauß, W. Hinrichs, I. Witt, P. Fromme, W. Saenger, in: N. Murata (Ed.), Research in Photosynthesis, Proc. Int. 9th Cong. Photosynth., Kluwer, Dordrecht, 1992, pp. 521–528.
[10] N. Krauß, W. Hinrichs, I. Witt, P. Fromme, W. Pritzkow, Z. Dauter, C. Betzel, K.S. Wilson, H.T. Witt, W. Saenger, Nature 361 (1993) 326–331.
[11] N. Krauß, W.-D. Schubert, O. Klukas, P. Fromme, H.T. Witt, W. Saenger, Nat. Struct. Biol. 3 (1996) 365–370.
[12] W.-D. Schubert, O. Klukas, N. Krauss, W. Saenger, P. Fromme, H.T. Witt, J. Mol. Biol. 273 (1997) 741–769.
[13] T. Yamaoka, K. Satoh, S. Katoh, Plant Cell Physiol. 19 (1978) 943–954.
[14] R.W. Castenholz, Methods Enzymol. 167 (1988) 68–92.
[15] P. Jekow, P. Fromme, H.T. Witt, W. Saenger, Biochim. Biophys. Acta 1229 (1995) 115–120.
[16] J. Hladik, D. Sofrova, Photosynth. Res. 29 (1991) 171–175.
[17] H.T. Witt, in: D.R. Ort, Ch.F. Yoccum (Eds.), Oxygenic Photosynthesis, 1996, Kluwer, Dordrecht, pp. 363–375.
[18] S. Jansson, B. Andersen, H.V. Scheller, Plant Physiol. 112 (1996) 409–420.
[19] J. Kruip, D. Bald, B. Hankamer, J. Nield, A.F. Boonstra, J. Barber, E.J. Boekema, M. Rögner, in: P. Mathis (Ed.), Photosynthesis: from Light to Biosphere, Proc. Int. Conf. Photosynth., 10th, Kluwer, Dordrecht, 3 (1995) 405–408.
[20] J. Kruip, P. Chitnis, B. Lagoutte, M. Rögner, E.J. Boekema, J. Biol. Chem. 272 (1997) 17061–17069.
[21] J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, Nature 318 (1985) 618–624.
[22] J. Deisenhofer, O. Epp, I. Sinning, H. Michel, J. Mol. Biol. 246 (1995) 429–457.
[23] C.H. Chang, D. Tiede, J. Tang, U. Smith, J. Norris, M. Schiffer, FEBS Lett. 205 (1986) 82–86.
[24] J.P. Allen, G. Feher, T.O. Yeates, H. Komiya, D.C. Rees, Proc. Natl. Acad. Sci. USA 84 (1987) 5730–5734.
[25] U. Ermler, G. Fritsch, S.K. Buchanan, H. Michel, Structure 2 (1994) 925–936.
[26] W.-D. Schubert, O. Klukas, W. Saenger, H.T. Witt, P. Fromme, N. Krauß, J. Mol. Biol. (1998) accepted.
[27] R.E. Blankenship, Photosynth. Res. 33 (1992) 91–111.
[28] W.F.J. Vermass, Photosynth. Res. 41 (1994) 285–294.
[29] P. Fromme, H.T. Witt, W.-D. Schubert, O. Klukas, W. Saenger, N. Krauß, Biochim. Biophys. Acta 1275 (1996) 76–83.
[30] H. Käß, Dissertation, TU Berlin, Germany, 1995.
[31] L. Yu, I.R. Vassiliev, Y.-S. Jung, D.A. Bryant, J.H. Golbeck, J. Biol. Chem 270 (1995) 28118–28125.
[32] L. Yu, D.A. Bryant, J.H. Golbeck, Biochemistry 34 (1995) 7861–7868.
[33] A. Kamlowski, A. van der Est, P. Fromme, D. Stehlik, Biochim. Biophys. Acta 1319 (1997) 185–198.
[34] E.T. Adman, L.C. Sieker, L.H. Jensen, J. Biol. Chem. 251 (1976) 3801–3806.

[35] A. Kamlowski, A. van der Est, P. Fromme, N. Krauß, W.-D. Schubert, O. Klukas, D. Stehlik, Biochim. Biophys. Acta 1319 (1997) 199–213.
[36] R. Bittl, S.G. Zech, J. Phys. Chem. B101 (1997) 1429–1436.
[37] S.A. Dzuba, H. Hara, A. Kawamori, M. Iwaki, S. Itoh, Y.D. Tsvetkov, Chem. Phys. Lett. 264 (1997) 238–244.
[38] R. Bittl, S. Zech, P. Fromme, H.T. Witt, W. Lubitz, Biochemistry 36 (1997) 12001–12004.